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Sexual Differentiation of the Central Nervous System

Neil J. MacLusky and Frederick Naftolin

Summary. Sexual differentiation of reproductive and behavior patterns is largely effected by hormones produced by the gonads. In many higher vertebrates, an integral part of this process is the induction of permanent and essentially irreversible sex differences in central nervous function, in response to gonadal hormones secreted early in development.

In many species marked sex differences in the control of endocrine function and behavior by the central nervous system (CNS) are an integral part of the reproductive process, including the recognition of a suitable sexual partner, in mating, and in the subsequent production and rearing of young. Sex differences in central nervous function represent the outcome of interactions between several different factors, among which the hormones secreted by the gonads are of paramount importance.

Current concepts of CNS sexual differentiation have their origins in a series of experiments, performed almost 50 years ago by Pfeiffer (*1*). His experiments with the laboratory rat showed that the expression of masculine patterns of pituitary gonadotropin secretion in adulthood depended on factors released from the testes during early postnatal life. Thus, the development of masculine patterns of gonadotropin secretion could be induced in genetic females by transplantation of a testis into the neck shortly after birth whereas castration of genetic males at birth resulted in the development of characteristically feminine patterns of gonadotropin release (*1*). With the later demonstration that the functions of pituitary are regulated by the hypothalamus, it became clear that the testes must influence the development of centers located within the brain (*2*).

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Many other sexually differentiated neuroendocrine functions and behaviors are also dependent on early gonadal secretions. A general hypothesis has been formulated for the mechanism of CNS sexual differentiation which has much in common with the model for differentiation of the peripheral reproductive tract (*3*). The intrinsic pattern of CNS development is assumed to be organized along lines that are appropriate for the homogametic sex. In the heterogametic sex, differentiation away from this pattern occurs as a result of hormones produced by the gonads. Thus, in mammals the intrinsic pattern is female, with differentiation toward masculine patterns of gonadotropin secretion and behavior occurring in the male as a result of exposure to testicular hormones during development (*4*). In birds the homogametic sex is male, and differentiation of the female CNS phenotype occurs as a result of exposure to ovarian hormones (*5*).

This simple mechanism is not the sole determining factor in sexual differentiation of the CNS. In many cases, however, there is good evidence that early hormonal experience makes at least some contribution.

Role of Early Gonadal Hormone Secretions

Although sex differences in CNS function exist in a great many vertebrate phyla, it is only in birds and mammals that these differences can be attributed to early gonadal hormone secretions. In fishes and amphibia, there are species in which early exposure to gonadal steroids induces complete sex reversal (*6*); but there is insufficient evidence to ascertain whether these effects involve a permanent developmental change in the CNS, or if they reflect primarily hormone-induced differentiation of peripheral structures.

The effects of early gonadal hormone secretions on mammalian and avian CNS function are extensive and diverse (Table 1). In addition to reproductively oriented functions, such as sex behavior and the control of gonadotropin secretion, sex differences in a large number of other behavioral and neuroendocrine end points to some extent depend on early gonadal hormone secretions.

The diversity in the developmental effects of gonadal hormones raises the question of whether one or many different hormone-sensitive mechanisms are involved in CNS sexual differentiation. Although we cannot answer this question definitively, there is increasing evidence that separation of different developmental responses to gonadal hormones can occur. Species differ in the extent to which CNS functions are influenced by early gonadal hormone exposure. In rodents, early exposure to androgens from the developing testes results in permanent suppression of the capacity to support cyclic feminine pat-

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terns of gonadotropin secretion and instatement of the tonic release pattern characteristic of the male (*2*). There is no evidence for a similar early androgen-induced differentiation of the mechanism regulating pituitary function in the rhesus monkey or man (*7, 8*). Within species, some resolution of sexually differentiated CNS functions can be achieved. In mammals, it is now recognized that masculine CNS differentiation includes (i) suppression of the behavioral and neuroendocrine patterns characteristic of the female ("defeminization") and (ii) enhancement of the patterns characteristic of the male ("masculinization") (*9*). In a number of physiologic and pharmacologic conditions these processes can apparently occur independently (*10*).

How the effects of early gonadal hormone secretions on CNS development are expressed depends on several factors.

1) *Genetic factors*. There may be differences between species in the neural substrate on which the hormones act, in terms of both hormone sensitivity and the function of specific hormone-sensitive structures. In the rat, afferent connections from structures outside the mediobasal hypothalamus (MBH) are essential for the maintenance of normal cyclic ovarian function (*11*). In the rhesus monkey, there is a lesser dependence on extrahypothalamic input; thus complete deafferentation of the MBH does not abolish the capacity of female rhesus monkeys to support cyclic patterns of gonadotropin release (*8*). Important genetic differences can also occur between members of the same species. In the extreme case, a genetic defect may result in complete loss of sensitivity to a particular gonadal hormone, as with the Tfm (testicular feminized male) mutation (*3*). More subtle gene effects may take the form of strain differences in sensitivity to hormones (*12*) or changes in the nature of the response to early hormone exposure (*13*).

2) *Hormonal effects in adulthood*. These "activational" effects of the hormones differ from the earlier developmental or "organizational" effects in that they are not permanent but are reversed in the absence of hormones. In many animals, the expression of sexually differentiated reproductive behavior is absolutely dependent on appropriate circulating hormone levels. If the hormones are removed (for example, by gonadectomy), the behavior declines and can only be restored by replacement hormone therapy. Other sexually differentiated CNS functions vary in the extent to which they depend on the activational effects of gonadal hormones. There are, however, relatively few end points that are organized by gonadal hormones while remaining independent of later activational effects (*10*).

Table 1. Some mammalian CNS functions are subject to organizational effects of early gonadal hormone exposure. Adapted from a more extensive summary of organizational and activational effects of gonadal hormones on nonreproductive behaviors in (*97*).

CNS function	Animal	References
Regulation of gonadotropin, prolactin secretion	Rodents, sheep	(*2, 4, 16*)
Reproductive behaviors	Rodents, ferret, sheep, dog, rhesus	(*4, 9, 17*)
Nonreproductive behaviors		
Activity		
Running wheel	Rat	(*86*)
Open field	Rat, hamster	(*87*)
Intraspecies aggression	Rat, mouse	(*88*)
Play	Rat, rhesus monkey	(*89*)
Taste preferences	Rat	(*90*)
Scent marking	Gerbil	(*91*)
Feeding and body weight	Rat	(*92*)
Learning		
Active avoidance	Rat	(*93*)
Maze learning	Rat	(*94*)
Pituitary regulation of liver androgen metabolism via "feminotrophin" secretion	Rat	(*95*)
Circadian rhythms	Rat, hamster	(*96, 97*)
Response to brain lesions		
Septal area	Rat	(*98*)
Globus pallidus	Rat	(*99*)
Ventromedial hypothalamus	Rat	(*100*)
Orbital frontal cortex	Rhesus monkey	(*101*)

3) *Extrinsic influences from the environment and from social and learning experience*. The impact of the environment is perhaps most obvious in species that have evolved seasonal breeding patterns. In such animals, hormone-induced sex differences in neuroendocrine function or behavior may be apparent only at certain points during the year, or under appropriate artificially controlled environmental conditions. In some birds, choice of an appropriate mate is strongly influenced by experiences that occur soon after hatching. Thus, cross-fostering of eggs from one species to parents of another may result in "sexual imprinting" of the young chicks, so that in later life their mating preference is directed toward the phenotype of the foster parent (*14*). In many mammals (including rats, guinea pigs, and rhesus monkeys) early social deprivation impairs subsequent masculine sexual behavior (*15*).

Which Gonadal Hormones Are Involved?

The main testicular factor responsible for sexual differentiation of the mammalian CNS is probably testosterone, the major hormonal product of the developing testis. Treatment with testosterone can essentially substitute for the testis in masculinizing patterns of gonadotropin release and behavior (*3, 9, 16, 17*).

It would be premature, however, to conclude that sexual differentiation of the CNS is simply a function of the presence in males and absence from females of unbound circulating testosterone. Systematic radioimmunoassay measurements have shown that, in the laboratory rat, androgens circulate in females as well as males during the period when sexual differentiation of the CNS is believed to occur. The amount of androgen present is still a matter of controversy: some reports have indicated consistently higher testosterone concentrations in males than in females during early postnatal life (*18*), whereas others have shown a considerable overlap in the amounts present in the two sexes (*19*). In a detailed study of plasma testosterone concentrations in rats killed between days 17 and 23 after conception (birth on day 21) Weisz and Ward found that only on day 18 were the values obtained from males consistently higher than those from females (*20*). In spite of the difference in results, these reports suggest that there may be significant levels of androgen in both sexes during early development. The partial data available for other mammalian species are consistent with the idea that, although there do appear to be periods during early development when circulating androgen levels are higher in males than in females, this difference is far from absolute and in many cases is not sustained (*21*).

The role of androgen in the female remains unclear. Brief exposure to high levels of testosterone may sensitize the developing male CNS to the effects of

Table 2. Relation between the length of gestation and the timing of developmental critical periods for CNS sexual differentiation.

Animal	Gestation or incubation (days)	Critical period* (after conception)
	Mammals	
Rat	20 to 22	18 to 27 days
Mouse	19 to 20	Postnatal
Hamster	16	Postnatal
Guinea pig	63 to 70	30 to 37 days
Ferret	42	Postnatal
Dog	58 to 63	Prenatal + postnatal
Sheep	145 to 155	~30 to 90 days
Rhesus monkey	146 to 180	~40 to 60 days
	Birds	
Japanese quail	17 to 18	Prehatching
Domestic chicken	22	Prehatching
Zebra finch	12 to 14	Posthatching
Pigeon	14	Posthatching

*Figures are given only for those species in which the duration of the critical period has been systematically studied. For the other species listed, some doubt remains as to the precise timing of the period of CNS sensitivity to gonadal hormones. In these cases the information given (pre- or postnatal, pre- or posthatching) indicates when gonadal steroids have been shown to exert organizational effects on the CNS. The data are from (*10, 102*).

subsequent lower testosterone concentrations (*20*). Thus, sex differences in circulating testosterone might only be necessary when sexual differentiation begins: thereafter similar hormone concentrations could produce entirely different effects. There is evidence that low levels of androgen in female rodents at around the time of birth can lead to potentiation of feminine as well as masculine sexual behavior (*10, 22*). There may be hormonal factors in the female that serve to protect her from the differentiating effects of androgen. In rats the presence of the ovaries tends to inhibit the defeminizing effects of neonatal androgen treatment (*23*). Resko has suggested that in rhesus monkeys progesterone serves to protect developing female fetuses from circulating androgens, the major determinant of CNS differentiation being the circulating ratio of testosterone to progesterone, rather than simply testosterone concentrations (*24*). A similar mechanism has been proposed to operate in the rat (*25*), although controversy still exists over the question of whether there are sex differences in the progesterone content of serum from newborn rats (*20*).

Information regarding the hormones mediating avian sexual differentiation remains sparse. Avian embryos are capable of synthesizing estrogen (*26*); and in some birds this hormone seems likely to represent the ovarian product responsible for feminine differentiation. Administration of estrogen to developing male pigeons, chickens, or Japanese quail results in demasculinization of reproductive behavior patterns (*5, 27*); while pharmacologic blockade of estrogen action in female quail prevents demasculinization (*28*). This pattern, however, is probably not common to all avian sexually differentiated behaviors. In the female zebra finch treatment with estrogen soon after hatching induces the development of male courtship and singing behaviors, but in males similar treatment is without effect (*29*).

Developmental Periods of Hormone Sensitivity

In all species so far examined the CNS does not remain equally sensitive to the permanent organizational effects of gonadal hormones throughout early life. Instead, there is a developmental period for each species during which the CNS is more sensitive to these effects than at any other time. This period—commonly referred to as the "critical period" for sexual differentiation—has been delineated in four placental mammals (rat, guinea pig, sheep, and rhesus monkey) by examination of the effects of endogenous hormones and timed hormone treatments on sexually differentiated neuroendocrine and behavior patterns (Table 2).

The critical period is an empirical concept, and does not represent a clearly defined stage of development. Nor does it necessarily encompass the entire period during which gonadal hormones contribute to the organization of CNS function. In practice, the end points commonly used to define the critical period are those associated with the control of reproductive function and sex behavior. It cannot be assumed that all sexually differentiated CNS functions are maximally sensitive to gonadal hormones within the same period. Moreover, within the critical period itself there may be significant variability in the response of different end points to gonadal steroids. For example, in rats the mechanisms regulating cyclic secretion of gonadotropin and female sex behavior are most sensitive to androgen soon after birth and are relatively unaffected prenatally by androgen. Masculine sexual behavior, by contrast, is highly sensitive to androgen treatment before birth (*10*).

The available data suggest that the beginning of the critical period may follow differentiation of the testicular Leydig cells and the onset of testosterone secretion. In rats typical Leydig cells first appear between days 16 and 18 after conception, just before the presumed start of the critical period (*30*). In guinea pigs, testicular androgen production rises to a maximum at day 29 or 30 of gestation: in female guinea pigs organizational effects of prenatal androgen treatment first become apparent when the androgen is given on day 30 of gestation (*31*).

Morphological studies of the developing CNS have led to the hypothesis that maximal sensitivity to gonadal hormones may be associated with a particular stage of neuronal maturation. In the rat, many of the hypothalamic structures believed to be involved in sexual differentiation are poorly differentiated at birth (*32*). Studies on the effects of altered thyroid hormone status have shown that hyperthyroidism, which accelerates cerebral maturation, shortens the postnatal period during which the rat brain centers controlling gonadotropin secretion can be differentiated by treatment with low doses of androgen. In contrast, hypothyroidism prolongs this period (*33*). These results are consistent with the idea that the CNS may be most sensitive to androgen during some early phase of neural differentiation. A relation between the timing of the critical period and cerebral development may also exist in other species. Although the temporal relation between the critical period and the events of conception and birth is far from constant (Table 2), this is at least in part a reflection of interspecies variability in the stage of development at which birth occurs. In species that are relatively less mature at birth (such as the rat, hamster, and pigeon), the critical period extends into postnatal life; whereas in animals that are more fully developed at birth (such as the guinea pig, rhesus monkey, and quail), the critical period tends to be predominantly or entirely prenatal (*10*).

Although CNS function can be organized by a single exposure to gonadal steroids during a critical period, differentiation is not always complete within this

period. Full development of the response to early gonadal hormones may partly depend on subsequent hormonal experience. For example, female rats treated neonatally with a low dose of androgen exhibit a few normal estrous cycles after puberty and then spontaneously become anovulatory (*4, 34*). This "delayed anovulatory syndrome" (DAS) depends on the continued presence of the ovaries. Thus, if the ovaries are removed prior to puberty the animal retains the capacity to support cyclic ovarian function well beyond the time at which it would normally be lost. These results suggest that complete development of sex differences in patterns of gonadotropin release may require the presence of gonadal steroids after the end of the critical period. Analogous findings have been reported for sexual behavior in the japanese quail. Complete demasculinization of quail sexual behavior in the female is dependent on the presence of estrogen after hatching as well as in ovo (*35*).

Hormone Target Cells in the Developing Brain

The effects of androgen on the CNS appear to be exerted directly, and do not require mediation by peripheral tissues. Thus, both anovulatory sterility and masculinization of patterns of sex behavior can be observed in female rats given intracranial testosterone implants soon after birth (*36*). The precise location of the androgen-sensitive sites within the brain, however, remains somewhat uncertain.

Two basic strategies have been followed in attempting to identify target areas for gonadal hormones in the developing CNS. First, the effects of stereotaxic hormone implants have been examined. In female rats, implantation of androgen into either the hypothalamus or the preoptic area shortly after birth results in a change in the pattern of subsequent sexual development (*36*). Christensen and Gorski (*37*) have reported that the responses elicited from these brain regions are dissimilar. The predominant effect of testosterone implants in the dorsal preoptic area is an increase in both masculine and feminine sex behaviors, but in the ventromedial hypothalamus similar implants inhibit both female sex behavior and the development of the capacity to support cyclic ovarian function.

The second strategy used to localize the effects of gonadal hormones is based on autoradiographic identification of sites of radiolabeled hormone concentration within the developing brain. Although there is no guarantee that sites of gonadal steroid uptake are necessarily involved in CNS differentiation, autoradiography has proved to be of considerable value in identifying hormone target cells. In the neonatal rat, Sheridan and co-workers (*38*) have identified within the hypothalamus, preoptic area, and amygdala several areas that concentrate ^{3}H-labeled testosterone or its metabolites (Fig. 1). They observed strikingly similar distributions of labeled cells after administration of ^{3}H-labeled estradiol (*38*).

In the chick, Martinez-Vargas and coworkers have identified ^{3}H-labeled estradiol–concentrating neurons in the preoptic area and hypothalamus on day 10 of incubation in ovo (*39*). By the time of hatching, other structures including the amygdala, ventrolateral septum, olfactory tubercle, and regions of the mesencephalon are also labeled by ^{3}H-labeled estradiol.

Role of Androgen Metabolites: Aromatization Hypothesis

The importance of local metabolism in the mechanism of adrogen action on peripheral target tissues is well documented (*40*). Figure 2 illustrates the major pathways by which potentially active metabolites of testosterone are formed within the neonatal rat brain. There are essentially two pathways to be considered: the first involves 5α-reduction of the C4-C5 double bond to give 5α-dihydrotestosterone (5α-DHT) which can then be further reduced at the 3 position to give 3α- and 3β-androstanediol; the second involves aromatization of the A ring followed by hydroxylation at either the 2 or 4 position to yield the "catechol" estrogens (*41*).

Early exposure to estrogen affects mammalian feminine sexual development in much the same way as early exposure to testosterone. During the

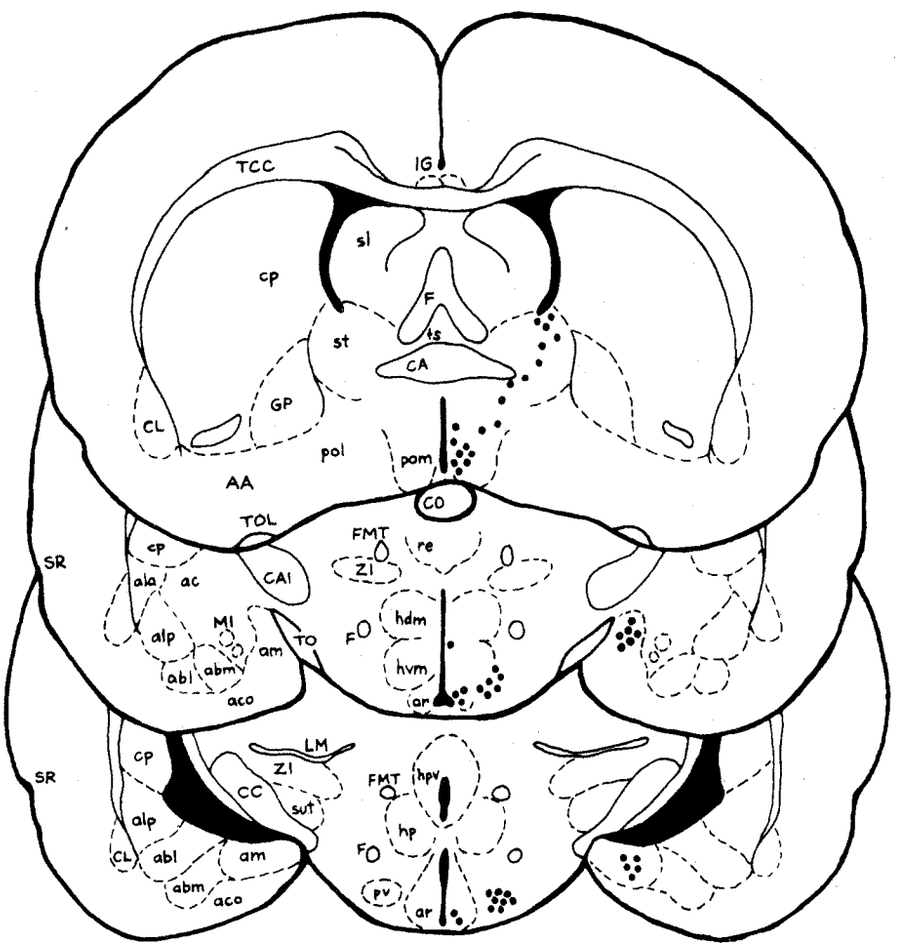

Fig. 1. Topographic distribution of neurons that concentrate ^{3}H-labeled testosterone or its metabolites in the newborn rat brain. The results of thaw-mount autoradiogram prepared from rats injected with ^{3}H-labeled testosterone are presented schematically in coronal sections through the preoptic area, central hypothalamus, and central amygdala. Areas with dots on the right-hand side of the figure represent accumulations of radioactively labeled neurons. Abbreviations for labeled cell groups: *aco*, nucleus amygdaloideus corticalis; *am*, nucleus medialis amygdalae; *ar*, nucleus arcuatus; *hpv*, nucleus periventricularis hypothalami; *hvm*, nucleus ventromedialis hypothalami; *pom*, nucleus preopticus medialis; *pv*, nucleus premammillaris ventralis; *st*, nucleus interstitialis striae terminalis. [From (*38*); courtesy of Karger]

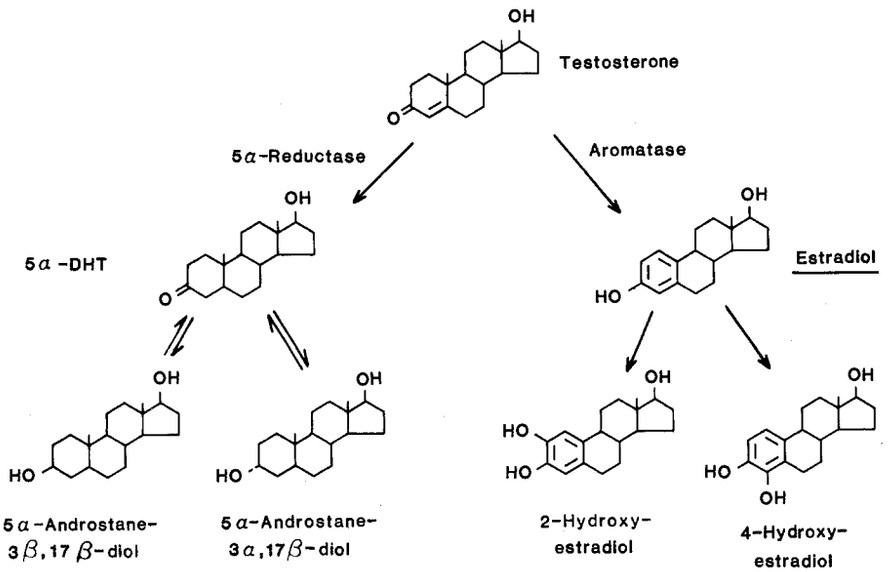

Fig. 2. Major possible routes of testosterone metabolism in developing brain tissue which result in the formation of physiologically active steroids (*10, 41, 46*).

1940's several workers reported that treatment of female rats with estrogen during gestation or shortly after birth resulted in a pattern of anovulatory sterility in adulthood which closely resembled that observed after perinatal testosterone administration (*42*). Subsequent work confirmed and extended these findings; many of the effects of testosterone on the developing brain were produced by estrogen (*43*). The full significance of these observations did not become apparent, however, until the early 1970's, when three observations brought about a complete reevaluation of the role of estrogen in sexual differentiation. First, 5α-DHT and other 5α-reduced androgens were shown to be far less effective than either testosterone or estradiol at inducing defeminization of the neonatal rat brain (*44*). Second, the effects of testosterone treatment in neonatal female rats could be blocked by administration of the estrogen antagonist MER-25 (*45*). Third, the developing brain itself is a site of androgen-to-estrogen conversion (*46*). These findings indicated that estrogen formation might play an important part in mediating the developmental effects of testosterone.

We now know that, in the rat, local estrogen formation plays a crucial role in sexual differentiation of the brain. Selective pharmacologic inhibition of estrogen formation from androgen or of the interaction between estrogen and its receptor sites dramatically impairs the developmental response of the CNS to perinatal testosterone (*47*). Studies with synthetic estrogens have established that the developing rat brain is exquisitely estrogen-sensitive: the minimum dosage required to produce defeminization of behavior and gonadotropin secretion in neonatal female rats is considerably lower for estrogens than for testosterone. Particularly compelling evidence for the role of aromatization has come from studies of Tfm male rats, which have severely reduced levels of androgen receptors compared to their normal siblings but show normal CNS levels of both estrogen receptors and aromatase (*48*). If androgen-specific receptor mechanisms played an indispensable role in the actions of testosterone on the developing brain, Tfm male rats would not be expected to undergo brain sexual differentiation. In fact, this does not appear to be the case. Patterns of gonadotropin release and sex behavior in Tfm male rats do differentiate under the influence of testicular secretions (*49*).

It would be premature, however, to conclude that testosterone and the 5α-reduced androgens play no direct role in CNS sexual differentiation. Several observations seem inconsistent with the idea that androgen acts entirely via conversion to estrogen. Testosterone-induced sexual differentiation is inhibited by androgen antagonists as well as estrogen antagonists (*50*). In male rats castrated at birth the developmental effects of systemic low-dose estrogen treatment seem to be potentiated by the simultaneous administration of 5α-DHT (*51*). Although sex behavior in Tfm male rats is clearly differentiated toward the masculine phenotype, the saccharin preference of these males remains female (*52*).

In other mammalian species there is a good deal of variability in the extent to which estrogen is required for sexual differentiation of the CNS. In female guinea pigs and rhesus monkeys, the expression of masculine sex behavior can be potentiated by prenatal treatment with 5α-DHT. Suppression of female sex behavior and cyclic ovarian function in guinea pigs, by contrast, may involve aromatizable androgens (*10*). The hamster seems very much like the rat in that both the masculinization and defeminization aspects of sexual differentiation can be induced by either aromatizable androgens or estrogen (*53, 54*).

The role of the other androgen and estrogen metabolites remains to be established. The effects of the 3α- and 3β-androstanediols are difficult to investigate critically, in view of the possibility of back conversion of these compounds to 5α-DHT. With regard to the catechol estrogens, we do know that both the 2- and 4-hydroxy estrogen derivatives are capable of defeminizing patterns of gonadotropin release when injected into neonatal female rats [4-hydroxyestradiol being at least as potent as estradiol with regard to this end point (*55*)]. It remains uncertain, however, whether this indicates any *specific* involvement of catechol estrogens in sexual differentiation.

α-Fetoprotein and the Protection Hypothesis

In placental mammals, the fetus is continually exposed to endogenous estrogen from the placenta and maternal circulation. If estrogen formation within the brain plays a vital role in sexual differentiation, then it follows that the fetus must somehow be protected from the effects of circulating estrogen. In rats and mice, the mechanism by which this protection is achieved is well established. In these two species, the immature brain is functionally protected from circulating estrogen by a plasma estrogen binding system. The developing yolk sac and fetal liver synthesize an estrogen binding protein (fetoneonatal estrogen binding protein, or FEBP) which circulates at high concentrations during the latter part of gestation and then gradually disappears over the first few weeks of postnatal life (*56*). This protein, which is immunochemically indistinguishable from the plasma α-globulin, α-fetoprotein, binds and effectively sequesters much of the estrogen present in the fetal and neonatal circulations. Significantly, however, it does not bind testosterone: hence testosterone is free to enter the brain where it can be converted to estrogen and interact with cellular estrogen receptors (Fig. 3).

Several experimental observations confirm the effectiveness of the FEBP protection mechanism. Although the levels of estradiol in the blood of the neonatal female rat are high, free estradiol does not seem to be readily available in the tissues (*57*). The administration of antibodies to α-fetoprotein newborn female rats produces effects on sexual development that resemble those of estradiol injections (*58*). Particularly compelling evidence has come from studies with synthetic estrogens. As indicated in Fig. 3, synthetic estrogens such as diethylstilbestrol (DES) and Ru 2858 exhibit markedly lower affinities than estradiol for FEBP. As might be expected if FEBP does indeed serve to protect the developing tissues, DES and Ru 2858 are more potent than estradiol in inducing sexual differentiation of female rat brain (*59*).

It is not yet possible to extend the FEBP protection hypothesis to species other than the rat and mouse. Although α-fetoprotein is present in many developing vertebrates, the ability to bind estrogen seems to be a property of a particular α-fetoprotein variant. Indirect evidence, based on the relative potencies of estradiol and Ru 2858, suggests that the situation in the hamster may be similar to that in the rat (*54*). In contrast, in humans only a small proportion (~ 0.1 percent) of circulating α-fetoprotein binds estrogen (*60*), while in guinea pigs attempts to demonstrate a specific fetal blood-borne estrogen binding system have so far been unsuccessful (*61*).

However, elements of the aromatization-estrogen response mechanism are clearly present in guinea pig and human fetuses. As was mentioned earlier, guinea pig sexual development is sensitive to prenatal exposure to estrogens, as well as androgens. The human fetal brain is capable of converting androgen to estrogen (*46*). There is no a priori reason to suppose that aromatization does not play a part in the response of the developing human or guinea pig CNS to prenatal androgen. Yet, in both man and guinea pig, we apparently cannot invoke α-fetoprotein as a mechanism for the regulation of free circulating estrogen levels during gestation.

The cellular localization of α-fetoprotein within the rodent brain has been studied. α-Fetoprotein is present in the neonatal rat brain as well as in the bloodstream (62). It is not merely an extracellular component of immature brain tissue, but is in part localized within developing nerve cells. Ths localization exhibits a striking regional specificity: those regions of the hypothalamus, preoptic area, and amygdala that concentrate ^{3}H-labeled testosterone and ^{3}H-labeled estradiol from the circulation are apparently devoid of intracellular immunoreactive α-fetoprotein (*63*). These observations suggest that the rat and mouse FEBP–α-fetoprotein systems may have more than just a protective role—possibly serving as a modulator of intracellular estrogen rather than as a strict barrier to entry of the hormone (*32, 63*).

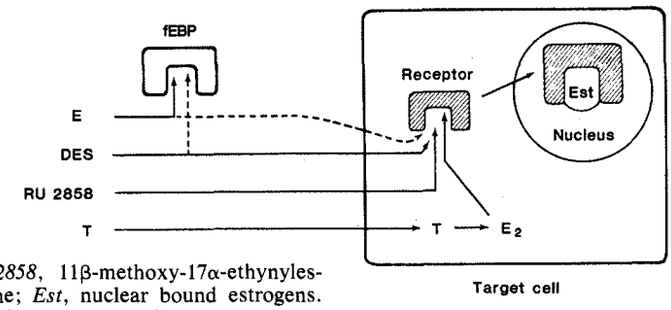

Fig. 3. Schematic diagram of the protective role of fetoneonatal estrogen binding protein (*fEBP*) in neonatal rats, and the ability of synthetic estrogens and testosterone to bypass this mechanism. Abbreviations: E_2, estradiol; *DES*, diethylstilbestrol; *Ru2858*, 11β-methoxy-17α-ethynylestradiol; *T*, testosterone; *Est*, nuclear bound estrogens. [From (*68*); courtesy of *Brain Research*]

Steroid Receptors in the Developing Brain

The general model proposed for steroid hormone action involves an initial binding reaction between the hormone and a sterospecific cytoplasmic receptor site. Subsequently, the hormone-receptor complex moves to the cell nucleus, where it is bound to the chromatin, initiating a cellular response (*3, 40*). This type of receptor system is believed to mediate many of the activational effects of steroid hormones on CNS function (*64*). We now believe that the organizational effects of estrogens and aromatizable androgens on the developing rat brain are mediated through a similar receptor mechanism (Fig. 3).

Much of the evidence for this stems from studies in the rat. Putative cytoplasmic receptors for androgens, estrogens, and progestins have now been identified in brain extracts of the newborn rat (*65, 66*). Physicochemically, the receptors appear similar to the homologous receptors from mature brain tissue. Two factors, however, set the neonatal systems apart from those present in adulthood. (i) The tissue concentrations of receptor sites are not static, but change rapidly both during and after the perinatal critical period; (ii) the regional distribution of estrogen receptors within the brain changes during development. In adulthood, these receptors are concentrated in structures within the corticomedial amygdala, preoptic area, and mediobasal hypothalamus. In neonatal rats, however, a diffuse population of cells containing estrogen receptors is also found extending through layers 5 and 6 of the cerebral cortex (*67*). These cortical estrogen receptors decline to adult levels after postnatal day 10 (*68, 69*).

Autoradiographic and biochemical studies indicate that the androgen and estrogen can bind within cell nuclei in the developing rat brain. The biochemical data show that the estrogen binding systems are capable of interacting with estradiol synthesized locally from androgen. After the administration of ^{3}H-labeled testosterone to newborn rats, much of the radioactivity recovered from amygdaloid, preoptic area, and hypothalamic cell nuclei represents ^{3}H-labeled estradiol (although some labeled testosterone and 5α-DHT is retained) (*66*). Studies on the extent to which brain estrogen receptors are translocated into cell nuclei by endogenous gonadal steroids in newborn rats show higher cell nuclear receptor concentrations in males than in females. This sex difference is abolished by castration, or treatment with the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione) (*70*).

It seems likely that similar receptor systems are present in other species during early development. Cytoplasmic androgen and estrogen receptors have been identified in the mouse brain during perinatal life (*71*). Similarly, putative estrogen receptors have been identified in cytoplasmic fractions prepared from the brains of fetal guinea pigs (*61*). Autoradiographic studies (*39*) suggest that functional CNS estrogen receptor systems are probably present in the chick embryo well before hatching.

Biochemical Effects of Early Hormone Exposure

The cellular mechanisms that translate early exposure to gonadal steroids into a permanent developmental effect remain ill-defined. For the most part, what we know is based on indirect or circumstan-

Table 3. Androgen-dependent sexual dimorphism in the CNS. The data in this table are expanded from a similar summary in (*32*).

Sex Difference	Region	Animal	References
Neuronal nuclear and nucleolar size	Preoptic area, amygdala, ventromedial hypothalamus	Rat	(*103*)
Synaptic vesicles and terminals	Arcuate	Rat	(*104*)
Synaptic organization	Preoptic area	Rat	(*105*)
Dendritic branching patterns	Preoptic area		
	Suprachiasmatic nucleus	Hamster, rat	(*105*)
	Hippocampus (Ammon's horn)	Mouse	(*106*)
Gross nuclear volume	Preoptic area	Rat	(*107*)
	Lumbar spinal cord (5th and 6th segments)	Rat	(*77*)
	Nucleus robustus archistriatalis; nucleus hyperstriatum ventrale pars caudalis	Zebra finch	(*78*)

tial evidence. An important clue is provided by the similarity between the steroid binding systems present in the adult and developing brain. In mature tissues, the first step in the mechanism of response to steroid hormones is thought to be the initiation of changes in nucleic acid and protein synthesis (*3*, *40*). It seems reasonable to suppose that in the developing brain cell nuclear binding of steroid-receptor complexes may initiate similar biochemical events.

There is some evidence to support this hypothesis. In female rats, antibiotic inhibitors of protein and nucleic synthesis afford a degree of protection against the differentiating effects of early androgen injections (*72*). General effects of androgen on protein and RNA metabolism in the developing rat brain have been reported (*73*). It remains to be seen whether these effects reflect specific changes in cerebral macromolecular synthesis associated with sexual differentiation.

In the adult rodent brain an important consequence of gonadal steroid exposure is altered neurotransmitter function (*64*). There is some evidence to suggest that similar mechanisms may also operate during development. In 12-day-old rats, brain serotonin concentrations are higher in males than in females (*74*). This difference appears to be the result of perinatal androgen secretions. Several drugs that interfere with monoaminergic transmission (chlorpromazine, pargyline, reserpine) and an inhibitor of acetylcholinesterase (pyridostigmine) also influence sexual differentiation (*75*).

Insight into the way in which steroid effects on the developing CNS may ultimately be expressed has been provided by Toran-Allerand (*76*), who showed, using an in vitro culture technique, that estradiol and testosterone accelerate and enhance the outgrowth of neurites from explants of newborn mouse preoptic area and infundibular and premammillary regions of the hypothalamus. The effect is region-specific, other regions showing little or no response in culture to the same hormonal treatments. The hormonal specificity of the response is similar to that of androgen-induced CNS sexual differentiation. In contrast to testosterone, 5α-DHT has no measurable effect on neurite outgrowth in culture. Estrogen appears to be of primary importance: thus, the neuritic response can be blocked by adding either antibodies to estradiol, or the synthetic estrogen antagonist C1628 to the culture medium. These results suggest that the effects of estrogens and aromatizable androgens on the developing brain may involve a regional stimulation of neurite growth. This hypothesis assumes particular importance in view of the emerging evidence that early gonadal hormone exposure may actually induce structural alterations within the CNS.

Morphologic Sex Differences in the CNS

As Table 3 indicates, there is a growing list of examples of sex differences in CNS morphology. These differences fall into three general categories: (i) ultrastructural differences in cellular or synaptic organelles, (ii) differences in synaptic or dentritic organization, and (iii) differences in the gross volume of defined cell groups (Fig. 4). Many of these sex dimorphisms are at least partly dependent on early gonadal hormone secretions (*32*).

With two exceptions, neuroanatomic differences cannot be correlated with specific sexually differentiated CNS functions.

1) In the zebra finch (*Poephila guttata*) the avian song control system consists of a chain of five discrete brain nuclei, which are involved in the efferent motor pathway for singing behavior (*77*). In some birds the expression of male-like song depends on testicular androgen to the extent that castration inhibits and testosterone replacement restores male song patterns. Male singing is a function of gonadal hormone secretions soon after hatching, as well as androgen in adulthood. It has been shown that the CNS song control centers are larger in male zebra finches than in females (*77*); and for two of these centers the nucleus robustrus archistriatalis (RA) and the nucleus hyperstriatum ventrale pars caudalis (HVc), exposure to estrogen soon after hatching is required for development to occur along masculine lines (*29*). Estrogen treatment of newly hatched female finches results in a significant increase in the volumes of these two nuclei and, in addition, potentiates the response of the RA and HVc to androgens given in adulthood. These anatomic changes are paralleled by behavioral sensitivity to androgen such that females treated neonatally with estrogen show patterns of courtship and song behaviors in response to adult androgen treatment that closely resemble those of the normal male.

2) In male rats, testicular androgen released around the time of birth differentiates the complex of androgen-dependent penile spinal reflexes that are characteristic of male sexual behavior (*78*). In the spinal cords of male rats, a discrete group of androgen-concentrating motoneurons innervating the levator ani (LA) and bulbocavernosus (B) penile striated muscles have been identified and termed the spinal nucleus of the bulbocavernosus (SNB) (*79*). But in female rats, the LA and B muscles are either absent or vestigial; and the volume of the SNB is greatly reduced (*79*).

Mechanisms Involved in the Genesis of Morphologic Sex Differences

The mechanisms involved in the development of sex differences in CNS morphology remain unknown. Given the diversity of the structural differences which have been described, it is difficult to formulate a generally applicable hypothesis. One attractive possibility is that anatomic sex differences may represent an expression of growth-promoting effects of gonadal steroids, analogous to those observed in culture by Toran-Allerand (*76*). Acceleration and enhancement of the development of one component of the neural circuits involved in regulating reproductive function could

have far-reaching consequences in terms of the differentiation of postsynaptic cellular and dendritic component and competition for available postsynaptic space, as well as the specific formation of new synaptic contacts (*10*, *32*). Accelerated growth might bring about the survival of neural elements that would otherwise be eliminated competitively during later life. Several lines of evidence suggest that the survival of neurons may to some extent be dependent on the formation of synaptic contacts, cells that form from only a limited number of synaptic connections being preferentially eliminated during CNS maturation (*80*). Moreover, some loss of synaptic connections appears to be a normal feature of CNS differentiation (*81*). Gonadal steroids could alter this process, by selectively stabilizing some connections or enhancing the rate of degradation of others (or both).

Processes of enhanced growth and degeneration are not necessarily confined to early development. They may simply be more obvious during early life, as a result of the greater plasticity of the immature CNS. Thus, under some circumstances morphologic effects of gonadal steroid treatment can be demonstrated in the adult animal. Arai and coworkers have reported that estrogen treatment of neonatal female rats has a marked synaptogenic effect with respect to the formation of axodendritic synapses in the arcuate nucleus (*82*). This effect is not observed when estrogen is given in adulthood. If, however, the mediobasal hypothalamus is deafferentiated in adulthood (resulting in degeneration of a proportion of the presynaptic elements in the arcuate nucleus), the ability of estrogen to stimulate axodendritic synapse formation is restored. A similar effect may underlie reports that the behavioral response of adult male rats to septal lesions can be modified by postoperative treatment with estrogen (*83*). With respect to degenerative effects, estrogen treatment of adult female rats results within a few months in marked neuropathologic changes in the arcuate nucleus and loss of ovarian cyclicity (*84*). This may represent an exaggeration of a normal gonadal steroid-induced aging process: similar neuropathologic changes are observed in the arcuate nucleus of old (12 months), spontaneously acyclic rats; but, if ovariectomy is performed at 2 months of age the extent of the neuropathologic change at 12 months is reduced (*84*).

Our understanding of the way in which morphologic changes in the CNS are brought about would be greatly facilitated if a single defined group of sexually dimorphic neurons could be followed throughout development. This may soon become possible (*85*). Gorski *et al.* have described the development of the sexually dimorphic nucleus of the medial preoptic area of the rat (SDN-POA) (Fig. 4) from gestation through to adulthood (*85*). An increase in the size and number of neurons within the nucleus is apparent in the male starting at around the time of birth and continuing throughout the first 10 days of postnatal life. Interestingly, thymidine-labeled autoradiographic studies have shown that a small proportion of SDN-POA neuroblasts undergo final cell division as late as day 18 of gestation, when sex differences in circulating androgen are clearly apparent (*20*). Thus, it seems theoretically possible to specifically label neurons within the SDN-POA at the time of their final cell division and follow the morphologic effects of gonadal steroids on these cells into later life.

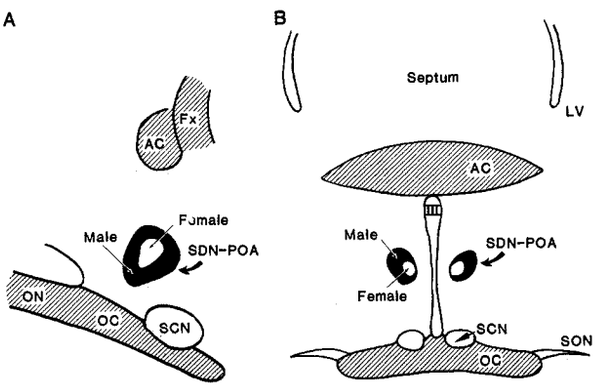

Fig. 4. Schematic localization of the sexually dimorphic component of the medial preoptic nucleus (*SDN-POA*) of the rat brain in the saggital (A) and coronal (B) planes. *AC*, anterior commissure; *Fx*, fornix; *LV*, lateral ventricle; *OC*, optic chiasm; *ON*, optic nerve; *SCN*, suprachiasmatic nucleus; *SON*, supraoptic nucleus; *III*, third ventricle. [(*107*); courtesy of *Brain Research*]

Conclusions

In the majority of species that have been studied the experimental data are consistent with the basic hypothesis for the mechanism of CNS sexual differentiation described. The hormonal products of the heterogametic gonad are of fundamental importance to the development of sex differences in CNS function. Thus, if the action of these hormones is prevented, sexual differentiation of the CNS is impaired.

This emphasis on the role of the heterogametic gonad does not necessarily mean that CNS development in the homogametic sex is entirely passive and independent of hormonal effects. Although the heterogametic sex in birds is female, song and courtship patterns in the zebra finch apparently differentiate in the male in response to early gonadal secretions (*29*). In mammals, the hormones released by the testis are important for masculine sexual differentiation; but this does not preclude the possibility that hormones may contribute to feminine CNS development. Factors such as progesterone may actively protect the female fetus from the influence of circulating androgen. Moreover, low concentrations of androgen or estrogen may actively promote the development of feminine behavioral traits.

The way in which gonadal steroids exert their effects on the developing CNS is not yet fully understood. Certain common features have emerged. Sensitivity to the differentiating effects of the hormones is high during early development. In many species, estrogen plays at least some part as either a circulating hormone or a locally active metabolite of circulating androgen. The initial step in the response mechanism may well involve a binding reaction between the hormones and cellular receptors, which closely resemble the receptors present in other steroid target tissues. Ultimately, the effects of early hormone exposure are expressed in terms of changes in the CNS at the structural as well as functional level.

A major unanswered question is how the initial reaction between the hormones and the developing CNS is translated into a permanent differentiating effect. Circumstantial evidence suggests that a hormone-induced change in gene expression may be involved, perhaps resulting in a cellular growth response. However, we really cannot be certain that this is the mechanism by which sexual differentiation occurs. For the most part, it remains impossible even to state where in the CNS the hormones act to bring about differentiation of particular behavioral and neuroendocrine functions, let alone to determine how these effects are produced. The introduction of in vitro and in vivo model systems for examining the response of specific cell groups to early gonadal hormone exposure offers valuable new approaches to this important remaining problem.

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108. This work was supported by an Alfred T. Sloan Foundation fellowship (to N.J.M.) and by grant HD 13587 from the National Science Foundation.

Neural Gonadal Steroid Actions

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Summary. Neurons sensitive to gonadal steroids are located strategically within neural circuits that mediate behaviors broadly related to the reproductive process. Some neuronal events and properties are regulated by these hormones. Variability in the occurrence and distribution of particular neural hormonal sensitivities across species may be related to variations in the hormonal requirements for sexual differentiation and for activation of reproductive behaviors.

To understand sex differences in behavior, we must understand the mechanisms that control these behaviors in adult life as well as the factors and mechanisms involved in their development. Because of the considerable degree to which environmental factors and learning play a role in behavior in our own species, investigators have turned to other species to study stereotyped behaviors as well as the underlying brain mechanisms. This has been a satisfactory approach for the study of behaviors regulated by hormones.

Goy has classified the sexually differentiated aspects of behavior into three categories: type 1, those that undergo differentiation under the influence of hormones and are also activated by hormones; and type 2, those that undergo differentiation independently of the influence of hormones but are activated by hormones; and type 3, those that are influenced by hormones during differentiation but are not activated by hormones (*1*).

For the most part, this article focuses on type 1 mechanisms, which include many of the components of reproductive behavior, broadly defined to include courtship, definition and defense of territory, and mating. I review what has been learned about the cellular mechanisms by which hormones activate behavior in a few species. I then consider some of the ways in which this information may be relevant to our understanding of the sexually differentiated features of the brain and behavior across species.

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Location of Neurons Sensitive to Gonadal Steroids in the Brain

Studies of hormone action on the brain at the cellular level have been facilitated by the localization of hormone-sensitive cell groups with biochemical and autoradiographic techniques. Estrogen, androgen, and, most recently, progestin receptors have been characterized and mapped within the brain (*2*). Much of this work has been done on the rodent brain, but we also have a good idea about receptor systems in brains of other mammals and of members of other vertebrate classes, as described below.

The map of estrogen-sensitive cells in the rat brain obtained by autoradiography (*3*) reveals clusters of estrophilic cells in the hypophysiotropic area as well as the corticomedial amygdala. The pattern of in vivo uptake and cell nuclear retention (*5*) of ^{3}H-labeled estradiol reflects this distribution (Fig. 1). Fewer and lesser labeled cells are found in regions such as the mesencephalic central gray and hippocampus (*3*).

Androgen-sensitive neurons are difficult to map owing to the fact that testosterone is extensively converted to estradiol (Fig. 1) as well as to 5α-dihydrotestosterone (DHT) in the brain (Fig. 2). Estradiol and DHT attach to estrogen and androgen receptors, respectively (Figs. 1 and 2). A problem in using DHT to study androgen receptors is that DHT is extensively metabolized when given systemically (*4*). However, enough DHT reaches the brain so that it is possible to obtain information about the distribution of androgen receptor sites; such studies have revealed a pattern of androgen-sensitive neurons (*5*, *6*) which overlaps to some extent with that of the estrogen-

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